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CHANGES OF ANTIOXIDANT ACTIVITIES OF BAKER'S AND ACTIVE DRY YEASTS DURING INCUBATION

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Abstract: The objective of the present study was to investigate changes of antioxidant activities of yeast autolysates obtained from three strains (two from baker's yeast, and one from active dry yeast) which has been incubated at 25°C and 37°C. Antioxidant activity has been measured at 24h, 32h, 48h, and 56h. Three different methods for measuring antioxidant activity has been used: radical scavenging activity assay against 2,2-diphenyl-1-picrylhydrazyl used as a source of free radicals, inhibition of Cu^{2+} induced lipid oxidation, and Fe^{2+} chelating activity. The active, dried yeasts developed higher antioxidant activity at 24h, but at the later measurement the rate of increasing of their antioxidant activity is significantly lower than of instant baker's yeasts. Some of the strains reached maximum of their antioxidant activities between 32 and 48h, until other continue to develop antioxidant activity, even at the end measurement point of the incubation.

INTRODUCTION

Free radicals may attack cellular structures, such as DNA, membrane lipids, and play a role in the pathology of numerous chronic diseases (Young and Woodside, 2001). Accelerated cell oxidation contributes to cardiovascular disease, tumour growth, aging, Alzheimer's disease and even affects the vitality and endurance of the body (Finkel and Holbrook, 2000; Radisky et al.,2005). Antioxidants are substances that delay or prevent the oxidation of cellular oxidisable substrates. They exert their effect by scavenging reactive oxygen species, activate detoxifying proteins, or preventing generation of reactive oxygen species (Halliwell 1997; Halliwell and Gutteridge, 1999.). In recent years, there was an increasing interest in finding natural antioxidants, capable to protect the human body (Kaur. and Kapoor, 2001; Miladi and Damak 2008.).

During the last five decades, special interest is directed toward yeasts because yeast constituents are considered as compounds of nutritional value to human and higher animals (Mogens et al, 2000). Yeast autolysates are concentrates of the soluble components of yeast cells, and they are generally produced by autolysis. In other words, an autolysates is the total content of the yeast following autolysis, which is essentially a degradative process carried out by activating the yeast's own degradation enzymes to solubilize the cell component found in the cell. Yeast autolysates are known under the name yeast extracts. Yeast extract comprises the water-soluble components of the yeast cell, the composition of which is primarily amino acids, peptides, carbohydrates, and salts. Nitrogen components and vitamins are the value of yeast because of their nutritional characteristics. The main applications of yeast extracts are in the food industry, as flavouring agent is soup, sauces, gravies, stews, snack food and canned food, as well as components in pet foods, cosmetic materials, and plant nutrients. Other applications include vitamin and protein supplements in health foods, and as a source of nutrients in microbiological media (Gorinstein et al., 1997; Harayama et al.1994; Hickenbottom 1996). They are mainly used in the fermentation industry as substrates and in the food industry as flavour improvers (flavouring agents) (Mogens, 2000).

In the literature, data regarding antioxidant activities of yeast autolysates from *Saccharomyces cerevisiae* are scarce. The antioxidant properties of yeast extract come from their contents of glutathione, Maillard reaction products and sulphur-containing amino acids (Ghiselli et al. 2000; Korch et all., 1991). Yeast cells and autolysates possess both enzymatic and non-enzymatic antioxidant systems, capable to defend against oxidative compounds as molecular oxygen, free radicals, and ions of transition metals (Beecher and Khachik, 1992; Moradas-Ferreira, 1996). Yeasts express levels of peroxisomal catalase A, which is important in the decomposition of the high amount of hydrogen peroxide resulting from fatty acid oxidation (Moradas-Ferreira, 1996). Glutathione is one non-enzymatic defence compound against oxidants. Because of the redox-active sulfhydryl group, which acts directly with strong oxidants, glutathione is an important free-radical scavenger (Moradas-Ferreira, 1996). In addition, glutathione is one of the metallothiones antioxidant compounds expressed by yeast to minimize the formation of hydroxyl radicals by ensuring the sequestration of transition metals, including copper and iron. The metallothiones have been linked to defence systems of metal-ion detoxification and homeostasis. Further, the metallothiones show capabilities to influence the formation and impact of both superoxide radical, and hydrogen peroxide, through magnesium, copper, and cadmium homeostasis. If the yeasts primary antioxidant defences do not overcome all the oxidants thrown at them, cells experience some degree of oxidative stress and produce secondary defence compounds (Moradas-Ferreira, 1996). Superoxide dismutase and thioredoxin peroxidase are two such

proteins. Antioxidative properties of superoxide dismutase from purified yeast extracts have the capabilities to inhibit the oxidation of emulsified linoleic acid, emulsified cholesterol, and ascorbic acid (Lingnert et all., 1989). Superoxide dismutase has likewise been connected to elimination of the superoxide radical. Use of reducing equivalents from yeast thioredoxin can reduce the concentration of hydrogen peroxide and hydroperoxides (Jeong, et all., 1998). Another antioxidant yeast protein, catalase T, is transcribed and produced after oxidative stress occurs from the formation of excess hydrogen peroxide, and acts directly to control its toxic effect. Apart from the yeasts internal antioxidant defences, during fermentation yeasts produce secondary metabolites, some of which could exhibit strong antioxidant activity.

The aim of this study was to investigate the antioxidant activities of autolysates obtained from strains S. cerevisiae from baker's and active dry yeast using various methods, and to follow changes in antioxidant defence capabilities during the incubation process.

MATERIALS AND METHODS

Chemicals: DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane], TRIS (2-Amino-2-hydroxymethyl-propane-1,3-diol), malondialdechide (1,1,3,3-Tetramethoxypropane), hydrochloric acid, acetone, and chloroform, were purchased by Sigma- Aldrich, Germany. 1-10 phenanthroline was purchased by Merk, Germany.

Yeast cultivation: 0,1 % solution of strains baker's yeast and active dry yeast were cultivated in 330 ml Saburo broth (Merck) for 24h,32h,48 and 56h at 37 and 25°C.

Preparation of yeast autolysates solutions of strains baker's yeast and active dry yeast were prepared as follows. After incubation, the cultural solution was centrifuged at 4000 rpm for 20min. The residues were re-extracted with deionized water, then centrifuged at the same rpm for 10 min. The obtained yeast autolysate solutions were combined in volumetric flask 500 ml and completed to mark with distilled water. The resulting autolysate solutions were used for analyses.

DPPH radical scavenging activity: Antioxidant activity was determined by modified method of Brand - Williams. In cuvette were added consequently 2 cm^3 DPPH solution (2.4mg DPPH in 100 cm^3 methanol), and 0.1 cm^3 autolysate. Simultaneously blank samples were prepared, were autolysate was substituted by distilled water. Three parallel measurements for each sample have been made. All cuvettes were placed in a dark box at temperature 25°C, for conducting reaction, and absorption at 515nm has been measured after 30 min incubation time. Calibration of method has been made with a water solution of trolox in concentration range between 100 and 500 μmol. l^{-1}. Results are calculated in units μmol trolox equivalent (μmolTE.kg^{-1}).

Fe^{2+} chelating activity: Ability of yeasts autolysates to chelate Fe^{2+} ions were investigated by measuring the masking effect of the autolysates during spectrophotometric determination of Fe^{2+}. In test tube 0.5 cm^3, 1.5 mmol/l $FeSO_4$, 5 cm^3, 10 mM/l TRIS-HCl (pH 7.4) in a saline solution, and 1 cm^3 from autolysate has been added. The tube is stirred, and left for 5 minutes at room temperature, and 0.3 cm^3 0.1% 1,10-phenanthroline solution has been added for conducting the colour reaction. The absorbance was measured at 510 nm in a spectrophotometer. Pure solvent was used instead sample, for blank measurement. Chelating activity was calculated as rate between the value of the reduction of measured concentration of Fe^{2+} ions, and a dry mass of yeast autolysate.

Inhibition of lipid oxidation: Ability of yeast autolysate to suppress lipid oxidation was investigated, by measuring the difference of concentration of TBARS in Cu^{2+} catalysed oxidation of solution artificial liposomes with and without addition of autolysate.

Extraction of egg phospholipids: Egg phospholipids were obtained from egg mélange by means of soxhlet extraction. 15 g egg mélange was put in the extractor. Chloroform/acetone mixture was used for solvent phase. After solvent regeneration, concentrated lipid solution was submitted on vacuum evaporation, remaining lipids were dissolved in acetone, and phospholipids were precipitated at 0°C. All purification procedures were conducted in inert atmosphere, under argon.

Preparation of artificial liposomes solution: 50 mg of egg phospholipids were mixed in a test tube with 10 cm^3 of 10 mM TRIS and 150 mM NaCl, pH 7,4. Multilamellar vesicles were generated by vigorous vortexing for 10 min at 3000 rpm. Procedure is repeated several times until sufficient amount of artificial liposomes solution is obtained. The solution is stored for a short time in Erlenmeyer flack under argon atmosphere with continuous stirring with magnetic stirrer. The artificial liposomes solution has been used in the day of preparation.

Analytical procedure: In 20 cm^3 test tubes sequentially were added 4 cm^3 artificial liposomes solution, 0.1 cm^3 from autolysate, and 0.1 cm^3 catalyzer solution. 0.1 M $CuSO_4$ was used as a catalyzer. The mixture was homogenized with vortex at 3000 rpm, and was incubated for 24h at 37°C. After incubation test tubes were cooled, and centrifuged in refrigerated centrifuge at 2500 rpm. 0.1 cm^3 from supernatant was added to 4 cm^3 from developing solution containing 0,375% thiobarbituric acid, 15% acetic acid, and 0,25 N HCl. The mixture was incubated at 95°C, and absorption at 540 nm was measured. 0.1 cm^3 10 mM TRIS-HCl in saline solution was used for blank sample. The method was calibrated with standard solutions developed at the same procedure, and containing between 6 to 60 μMol/l malondialdehyde in saline solution buffered at pH 7.4 with TRIS-HCl.

Inhibition of lipid oxidation was calculated as rate between the value of the reduction of measured concentration of TBARS, and dry mass of yeast autolysate.

RESULTS AND DISCUSSIONS

Free radicals are produced by normal growth of aerobic cells, and by impact from other external factors as toxins, radiation and other. In order to prevent oxidative damage and cell death, all aerobic organisms develop strong defence mechanisms against oxidative damage. They can be grouped in three main mechanisms. The first group is enzymatic antioxidant systems, which function is to decompose peroxides. The second group is metal chelators, which function is to bond pro-oxidant ions of transition metals in stable chelate complex. The third group is chain breaking compounds that are usually hydrogen donors or compounds with conjugated π-systems, capable to stabilize free radicals, and to break chain reaction. In this study enzymatic systems were deactivated with ethanol before analyses. Three methods were chosen for quantification of antioxidant activity. DPPH radical scavenging activity was chosen as fast and simple method for measuring chain breaking ability of the samples. The method is based on spectrometric measurement of concentration of stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is decreased under the influence of the measured sample. The method is calibrated by using trolox as standard antioxidant, and results are represented ad trolox equivalents. Fe^{2+} chelating activity was chosen as mean for quantifying the ability to bond ions of transition metals in stable chelate complex. The method is based on measuring masking effect of the sample onto spectrometric determination of iron by phenanthroline complex. Decreasing of absorbance at 510nm (specific wavelength, where iron-phenanthroline complex absorb light) is measured for Fe^{2+} chelating activity of the sample. For more complex studies combining both chelating and chain breaking activity method for measuring inhibition of lipid oxidation in models of natural lipid systems was chosen. We used a solution from artificial liposomes constituted by egg phospholipids in TRIS-HCL buffered saline solution. Oxidation was catalysed by Cu^{2+} ions. Concentration of TBARS was measured spectrometrically. Decreasing TBARS caused by the sample was used third and more complex criteria for antioxidant activity. Yeasts produce many different compounds serving as components in antioxidant defence systems. Most familiar of these are glutathione, sulphur containing amino acids (Hassan, 2011) and (1→3) -β-D-glucan (Jaehrig, et al, 2008). All these compounds have properties of chain breaking antioxidants. Glutathione, and sulphur containing amino acids are known as good chelators.

In this study two *S. cerevisiae* strains from baker's yeasts (samples 1 and 2), and one from active dry yeast (sample 3) has been incubated at 25°C and 37°C. Antioxidant activity has been measured on yeast autolysates at 24h, 32h, 48h, and 56h, and on inoculate.

Radical scavenging activity:

The results of the DPPH assay for extracts from inoculate and yeasts autolysates are shown in table 1.

Table 1. Change of DPPH scavenging activity during incubation of yeasts.

h	Sample	24h		32h		48h		56h	
		m (g)	AOA (µmol/kgTE)	m (g)	AOA (µmol/kgTE)	m (g)	AOA (µmol/kgTE)	m (g)	AOA (µmol/kgTE)
25°C	1	0,16	0,05	0,28	1,73	0,34	1,93	0,37	1.9
	2	0,19	0,44	0,29	0,96	0,32	1,1	0,35	1.44
	3	0,25	0,65	0,29	1,27	0,36	7,96	0,39	1,85
37°C	1	0,14	0,1	0,31	1,04	0,43	2,05	0,75	3
	2	0,16	0,07	0,3	0,87	0,47	1,85	0,77	1,8
	3	0,21	0,15	0,28	0,75	0,35	1,27	0,38	1,8
Yeasts	1	0,73							
	2	1,1							
	3	2,65							

Yeast extracts showed antioxidant activity from the same order, compared to literature data (Jaehrig, et al, 2008). Active dry yeasts show three times higher TE comparing to baker`s yeasts. The difference can be explained with higher number of cells per unit of weight in active dry yeasts than baker`s yeasts. Differences between two types baker`s yeasts can also be explained by differences in technologies and strains. Under optimal condition (t°C, and differential media-"Saburo") autolysates from cultivated yeasts, show higher chain breaking activity than extracts from commercial yeasts. When we investigate the dynamic of antioxidant activity during fermentation, (figure 1, and 2) we observe higher activity at 37°C comparing to 25°C. At 25°C autolysates are reaching the maximum of their activities after 32h at all three strains, and beginning of decreasing their activity after 56h. At 37°C we observe different behaviour. The antioxidant activity of the autolysates did not reach maximum even at the end point of cultivation, and differences between all three samples are small.

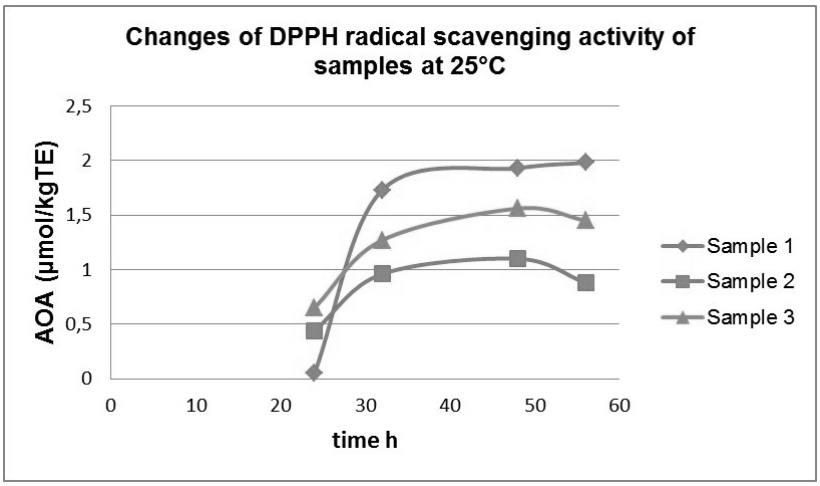

Figure 1. Changes of DPPH radical scavenging activity of samples 1,2 and 3 at 25°C.

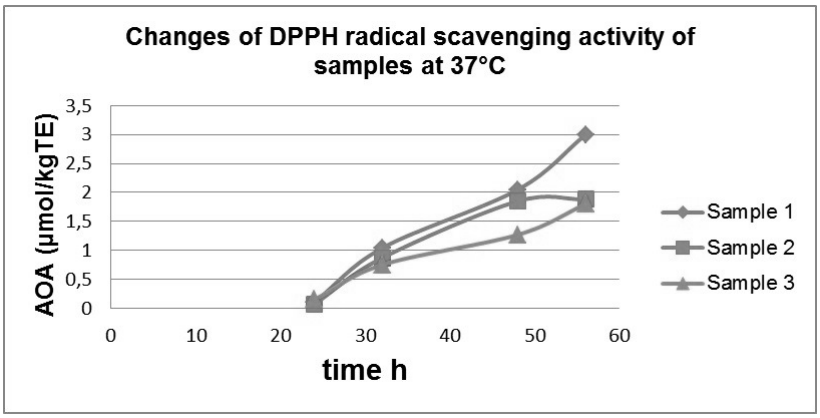

Figure 2. Changes of DPPH radical scavenging activity of samples 1,2 and 3 at 37°C.

Fe^{2+} chelating activity:

A significant mechanism for creating reactive oxygen species is catalysed by transition metals. Therefore, bonding transition metals in stable chelate complex is one of the important aspects of antioxidant activity. Results are represented in table 2 in relative units (ΔA/kg). We did not find in literature fully comparable data, regarding chelating activity of yeast extracts. Hassan shows data between 15 and 49% Fe^{2+} chelation on two different strains with dry weight between 2.5 and 6.25%. Yeasts from inoculates studied in our investigation showed between 9, and 10.4% Fe^{2+} chelation at 1% dry weight for baker`s yeasts, and 0.93% for active dry yeasts. Lower $Fe^{2+}$ chelation activity for active dry yeasts can be explained with lower content of chelators in active dry yeasts. Literature data support this conclusion, but at not so big extent. Mussati present glutathione content in baker`s yeasts between 10 and 30% higher than active dry yeasts (Musatti, 2013). When we study dynamics of antioxidant activity of yeasts autolysates (figures 3 and 4), we observe some similarities to radical scavenging activity. Chelating activity is reaching maximum around 32h. At 37°C we observe higher activity than 25°C. Autolysates from active dry yeasts reached a higher Fe^{2+}chelation activity than autolysates from baker`s yeasts. Differences between two strains of baker`s yeasts are statically negligible.

Table 2. Change of Fe^{2+} chelating activity during incubation of yeasts.

h	Sample	24h		32h		48h		56h	
		m (g)	AOA (ΔA/kg)	m (g)	AOA (ΔA/kg)	m (g)	AOA (ΔA/kg)	m (g)	AOA (ΔA/kg)
25°C	1	0,16	1	0,28	2.5	0,34	3.4	0,37	3.3
	2	0,19	0.74	0,29	3.24	0,32	3.66	0,35	3.6
	3	0,25	2,32	0,29	6,24	0,36	6,67	0,39	7,49
37°C	1	0,14	1,4	0,31	2,8	0,43	4,7	0,75	2,1
	2	0,16	1,6	0,3	2,9	0,47	4,6	0,77	2,1
	3	0,21	6,05	0,28	8,07	0,35	8,03	0,38	3,89
Yeasts	1	7.8 (10.4% Fe^{2+}chelation)							
	2	6.7 (8.9% Fe^{2+}chelation)							
	3	0.7 (0.93% Fe^{2+}chelation)							

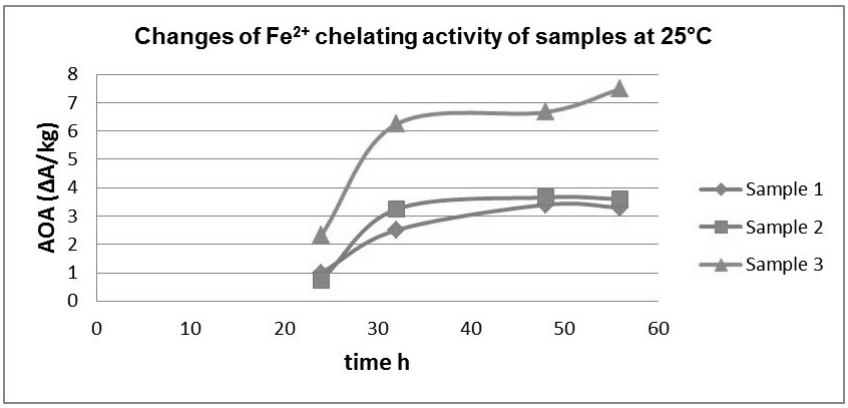

Figure 3. Changes of Fe^{2+} chelating activity of samples 1,2 and 3 at 25°C.

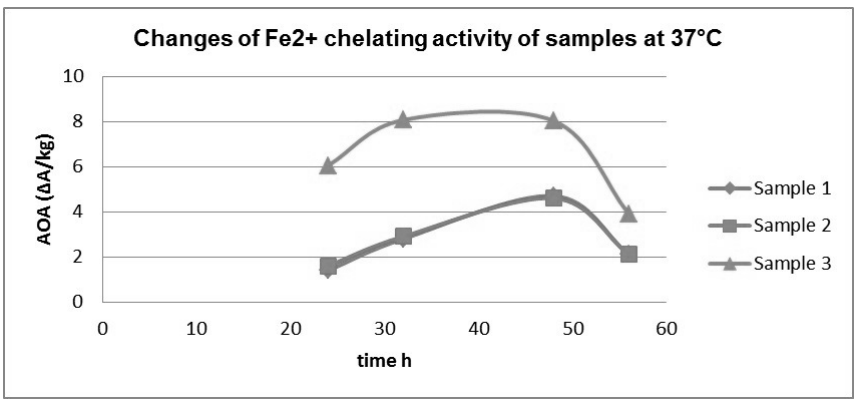

Figure 4. Changes of Fe^{2+} chelating activity of samples 1,2 and 3 at 37°C.

Inhibition of lipid oxidation:

One of the important mechanism of cell damage caused by reactive oxygen species is the oxidation of lipids in cell membranes. In order to investigate this effect, we developed procedure based on standard TBARS assay. By this method we investigate oxidation in a solution containing artificial lipid structures similar to cell membranes, caused by transition metal. Adding an antioxidant inhibit oxidation of the lipids and cause decreasing of TBARS in solution. We chose this method because it combines both chelating activity and chain breaking activity in one analysis. Results are represented in table 3 in relative units (ΔTBARS/kg). We could not find any article investigating inhibition of lipid oxidation caused by yeast autolysates. Our research shows 38% and 42% TBARS reduction at 1% dry weight for extracts from commercial baker`s yeasts, and 11% TBARS reduction from extract from commercial active dry yeast at 1% dry weight. Inhibition of lipid oxidation behaves very similar to chelating activity. After 32h of incubation, maximum of antioxidant activities is reached for all autolysates except autolysate

Table 3. Change of inhibition of lipid oxidation during incubation of yeasts.

h	Sample	24h		32h		48h		56h	
		m (g)	AOA (ΔTBARS/kg)	m (g)	AOA (ΔTBARS/kg)	m (g)	AOA (ΔTBARS/kg)	m (g)	AOA (ΔA/kg)
25°C	1	0,16	313	0,28	643	0,34	559	0,37	162
	2	0,19	316	0,29	695	0,32	710	0,35	57
	3	0,25	360	0,29	966	0,36	1083	0,39	51
37°C	1	0,14	429	0,31	549	0,43	116	0,75	27
	2	0,16	375	0,3	600	0,47	128	0,77	27
	3	0,21	524	0,28	821	0,35	1257	0,38	500
Yeasts	1	720 (42% reduction of TBARS)							
	2	650 (38% reduction of TBARS)							
	3	190 (11% reduction of TBARS)							

cultivated from active dry yeasts at 37°C which reach maximum at 48h. Changes of inhibition of lipid oxidation during fermentation can be observed on figures (5, and 6.).

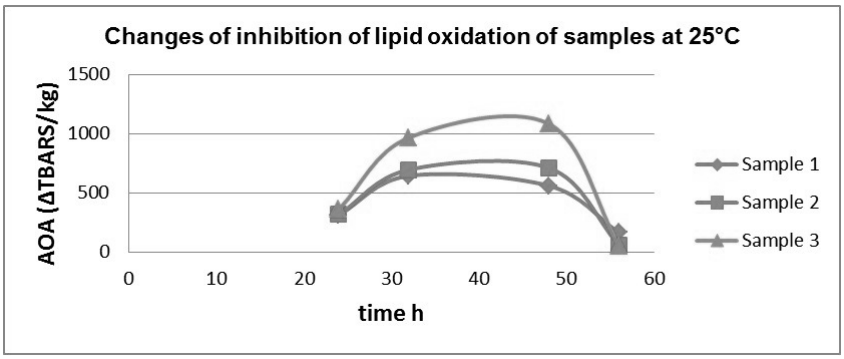

Figure 5. Changes of inhibition of lipid oxidation of samples 1, 2 and 3 at 25°C.

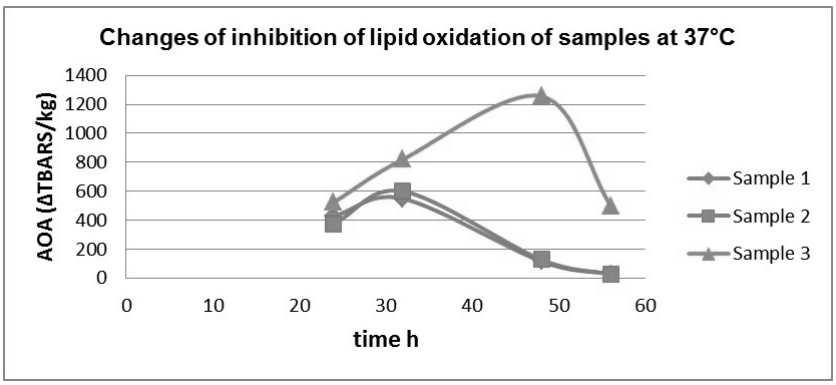

Figure 6. Changes of inhibition of lipid oxidation of samples 1,2 and 3 at 37°C.

Again, we observe close behaviour between two samples of instant bakers yeasts (sample 1, and 2), and significantly higher activity with active dry yeasts.

In general, we can see few tendencies. At first stage of incubation, we can see fast growing of antioxidant activity per unit of dry weight. At same point system is reaching a plateau, where antioxidant activities stop growing. In most of the samples that point is around 32h. At the second stage of incubation, antioxidant activity is changing with a low rate (top of the plateau). In cases where the end of this stage is observed, it is between 48 and 56h. Third stage of incubation is characterized with high rate of decreasing antioxidant activities.

Observation of different strains shows low differences between two samples from instant baker`s yeasts (samples 1, and 2), excluding DPPH assay. Sample 3 (active dry yeasts) behave differently. Autolysates from cultivated active dry yeasts show significantly higher antioxidant activity than autolysates from cultivated baker`s yeasts.

Incubation at different temperatures affects antioxidant activities of the autolysates. Strains incubated at 37°C reach higher antioxidant activity, than strains incubated at 25°C. Higher AOA could be caused by higher oxidative stress that unlock secondary defence mechanism.

When we compare incubated yeasts with inoculation, we observe similar or slightly higher inhibition activity with baker`s yeasts at their maximum and significantly higher with active dry yeasts. We could suppose that laboratory media (differential media-"Saburo") is much richer than used in industry, which is the reason for the higher antioxidant activity.

CONCLUSION

Aim of this study was to investigate dynamic of developing antioxidant activities in yeast autolysates during incubation. Studied yeast strains show high values of antioxidant activity. Selected strains showed the maximum of their antioxidant properties between 32 and 48h on incubation, when a relatively flat plateau of AOA activity can be observed. Strains incubated at 37°C show higher antioxidant activity than the one incubated at 25°C. At the end measurement point (56h) there are observable tendencies for decreasing of AOA.

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